

# ***TOPIC B – PART 7***

## ***ENZYMES***

IB Chemistry  
Topic B – Biochem



# (HL) B7 Enzymes - 3 hours

- B.7.1 Describe the characteristics of biological catalysts (enzymes). (2)
- B.7.2 Compare inorganic catalysts and biological catalysts (enzymes). (3)
- B.7.3 Describe the relationship between substrate concentration and enzyme activity. (2)
- B.7.4 Determine  $V_{\max}$  and the value of the Michaelis constant ( $K_m$ ) by graphical means and explain its significance. (3)
- B.7.5 Describe the mechanism of enzyme action, including enzyme substrate complex, active site and induced fit model. (2)
- B.7.6 Compare competitive inhibition and non-competitive inhibition. (3)
- B.7.7 State and explain the effects of heavy metal ions, temperature changes and pH changes on enzyme activity. (3)



# B7.1 – Biological Catalysts

- *B.7.1 Describe the characteristics of biological catalysts (enzymes). (2)*
- Enzymes are **globular** (functional) proteins that are specialized to catalyze biochemical reactions
  - Enzymes **increase the rate** of a chemical reaction without undergoing a permanent chemical change themselves
  - Provides an alternative mechanism with a **lower activation energy** for the reaction
  - The molecules that the enzyme works on are referred to as the **substrate**



# B7.1 – Enzymes

- The small part of the protein that allows for substrate binding is called the **active site**
- The enzyme combines **temporarily** to the substrate (via active site) to produce a having a lower free energy than that of an uncatalyzed reaction
- The **enzyme activity** is the **rate** at which a biochemical reaction takes place in the presence of an enzyme.
- **Measured** in terms of the rate of appearance of a product or consumption of the reactant.



# B7.1 - Enzymes

- Enzymes are specific to certain substrates and only a single type of reaction takes place without side reactions or by products.
  - Specificity occurs because
    - ◆ Active site has a very close fit to the substrate
    - ◆ Enzyme and substrate have complementary structures where all the charged (hydrophilic and hydrophobic) amino acids residues are paired.
    - ◆ Many enzymes are absolutely specific for a particular substrate (not even an enantiomer), where others will react with a whole class of molecules but at widely different rates.



# B7.2 – Inorganic v Biological (enzyme) Catalysts

- *B.7.2 Compare inorganic catalysts and biological catalysts (enzymes). (3)*
- **Enzymes (biological catalysts)** are very effective catalysts, functioning in dilute aqueous solutions, biological pH, moderate temperature
  - Activity and specificity depend on conformation or 3D structures (tertiary, quaternary).
  - Small changes in environment change the conformation and leads to activity changes.
- **Inorganic catalysts** can often be used in rather **extreme** conditions.



# B7.2 – Biological vs. Inorganic

- The major difference between enzymes (biological) and inorganic catalysts are shown below
- In general, enzymes are more specific and effective but only under ideal conditions

Enzymes	Inorganic catalysts
Enzymes are complex globular proteins	Inorganic catalysts are generally ions, or simple molecules
Enzymes are synthesized by living cells	Inorganic catalysts are not produced by living cells
Enzymes are usually highly specific in action	Inorganic catalysts are usually less specific in action
Enzymes are sensitive to changes in pH and temperature	Inorganic catalysts are usually less sensitive to changes in pH and temperature
Enzymes only function in aqueous solution	Some inorganic catalysts function in aqueous solution

Table 22.8 Differences between enzymes and catalysts

# B7.2 – Measuring Enzyme Activity

- Rate is determined by the amount of substrate that has been consumed, or the amount of product that has been formed.
- SI unit of rate is  $\frac{\text{mol}}{\text{dm}^3 \text{ s}}$  (  $\frac{\text{mol}}{\text{dm}^3 \text{ s}}$  ), but other units such as  $\frac{\text{mol}}{\text{dm}^3 \text{ min}}$  or  $\frac{\text{mol}}{\text{dm}^3 \text{ h}}$  are also used.
- A simple study can be done for the enzyme **catalase**, which catalyzes the break down of hydrogen peroxide to water and oxygen



■ **Catalase** is present in **all cells** and protects from hydrogen peroxide (byproduct of respiration)





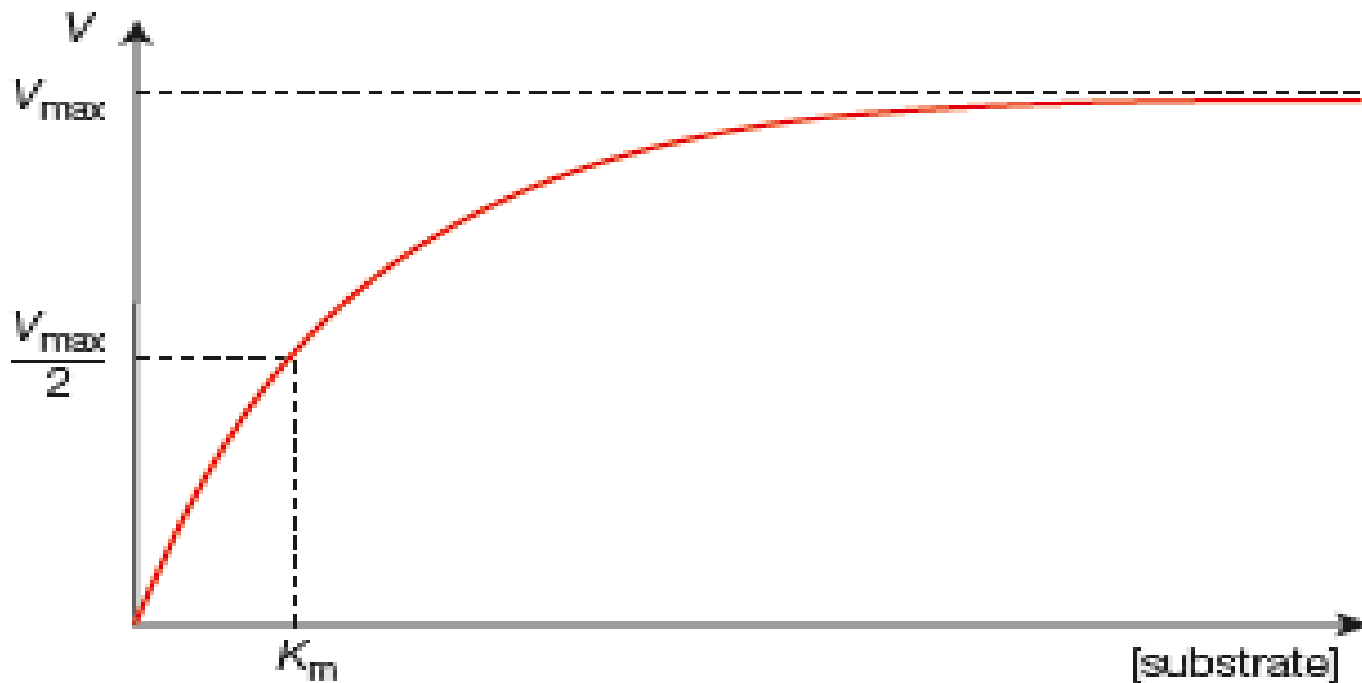
# B7.3 – Substrate vs Enzyme Activity

- *B.7.3 **Describe** the relationship between substrate concentration and enzyme activity. (2)*
- The general principles of reaction kinetics (topic 6) applies to enzyme-catalyzed reactions with one important feature not observed in non-enzymatic reactions
  - Saturation with substrate
    - ◆ At **low substrate** concentrations, the enzyme activity, **V (reaction rate)**, is proportional to substrate concentration, and reaction is **first order**
    - ◆ As substrate **concentration increases**, activity increases less and is no longer proportional, rate is now **mixed order**.



## B7.3 – Enzyme Activity

- As substrate **concentration increases further**, the activity tends to become independent of sub. conc. and approaches a constant rate,  $V_{\max}$ .
- In this region, rate is approximately **zero order** and is said to be saturated with substrate.



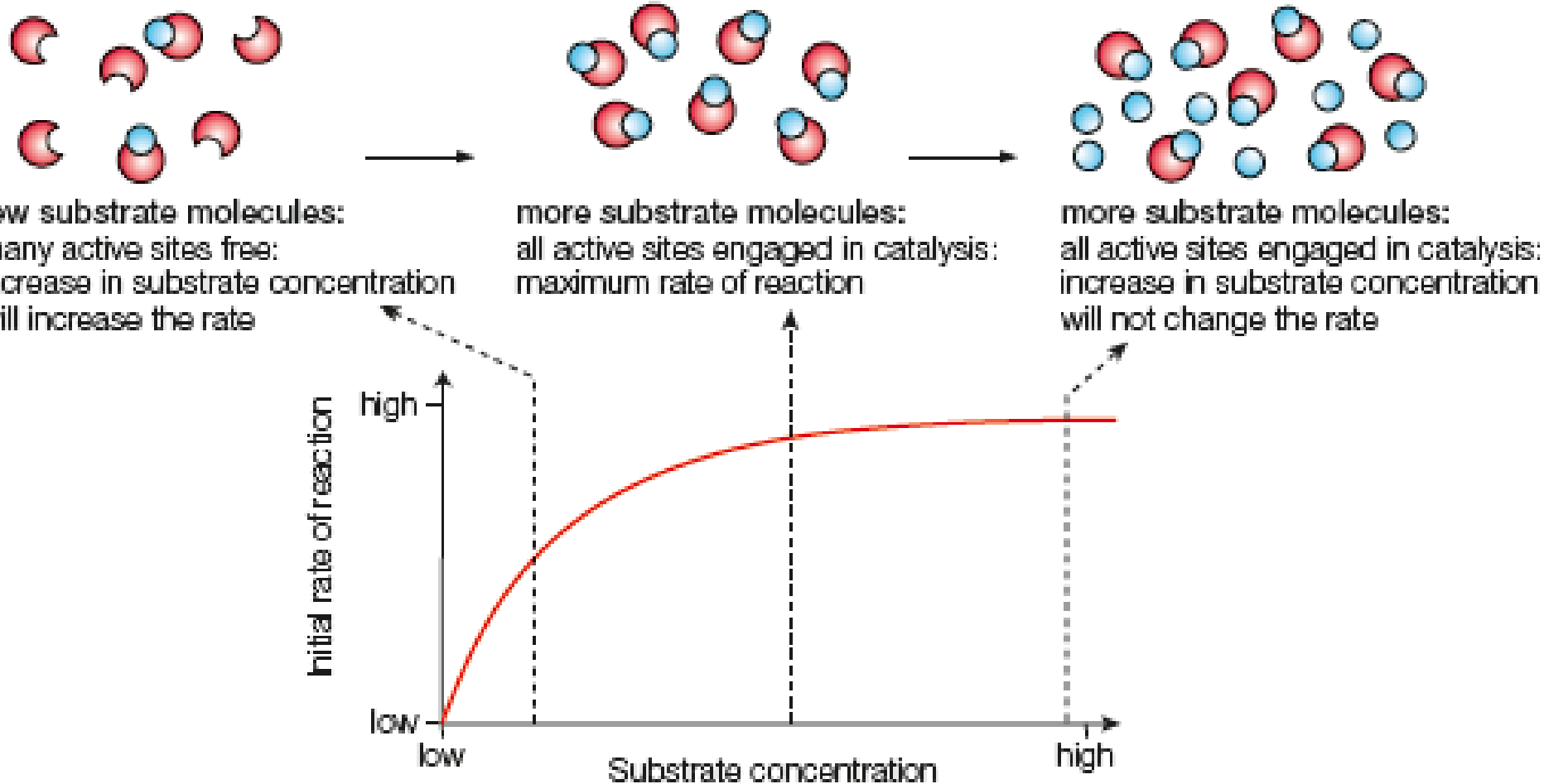
## B7.4 – Graphical Analysis

- *B.7.4 Determine  $V_{max}$  and the value of the Michaelis constant ( $K_m$ ) by graphical means and explain its significance. (3)*
- This saturation-based behavior suggests
  - That the enzyme and substrate react reversibly to form a complex as an essential step of the enzyme-catalyzed reaction
  - Enzymes possess active sites where the substrate binds and chemical reaction occurs
- Michaelis and Menten were the first researchers to develop a general theory of enzyme-catalyzed reactions and kinetics



# B7.4 – Substrate Conc. → Rate

- Analysis of substrate concentration and its effect on the rate of enzyme activity



## B7.4 – Michaelis Menten Theory

- The Michaelis Menten Theory (MMT) assumes that the enzyme, E, first binds with the substrate, S, to form an enzyme-substrate complex.
- This then breaks down to form the free enzyme and the product, P.
- Reactions are reversible and the enzyme-substrate concentrations is assumed to be constant during the reaction



## B7.4 – Values for MMT

- **Low [S]**,  $V$  increases almost linearly
- As **[S] increases** further,  $V$  increases rapidly
- Eventually,  $V$  reaches a limiting value called  $V_{\max}$  at **saturating [S]**
- **$V_{\max}$**  is the maximum activity at 'infinite' [S]
- The [S] at which  **$V_{\max}/2$**  is called the **Michaelis constant (MMC),  $K_m$**  (mol/L)
- The MMC,  $K_m$ , is therefore a measure of the affinity of an enzyme for its substrate



$K_m$  is not a fixed value and may vary with the structure of the substrate or the environment

## B7.4 – Specificity

- The catalytic properties and specificity of an enzyme are determined by the functional groups in a small region of the protein surface called the active site.
  - Active site found in **cleft or crevice**
  - Function of active site
    - ◆ Binding to substrate
    - ◆ Catalysis
- The **lock and key model** demonstrates the specificity of an enzyme
  - Lock (enzyme) and Key (substrate)



# B7.5 – Enzyme Action Mechanism

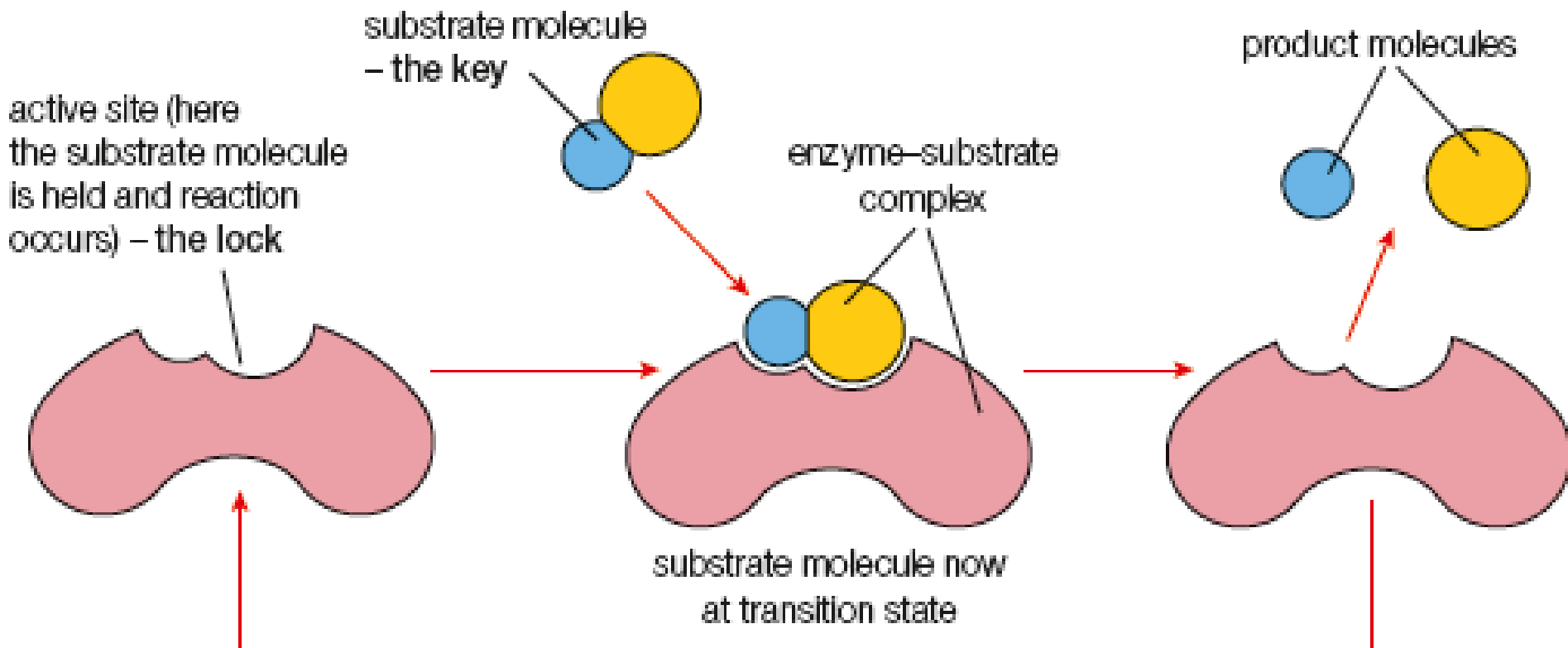
- *B.7.5 Describe the mechanism of enzyme action, including enzyme substrate complex, active site and induced fit model. (2)*
- As previously described enzymes function as catalysts by binding to their substrate molecule(s) at a specific pocket or cleft in the enzyme.
  - Binding site is known as the active site and is where the catalysis occurs (as well as inhibition)
    - ◆ Contains specific amino acids (AA) residues which are responsible for the substrate specificity and catalysis
    - ◆ AA's often act as proton donors or acceptors (zwitterion function from Part B - proteins).





# B7.5 – Enzyme Action

- Activity and specificity of many enzymes is explained by the lock and key hypothesis
- $E + S \rightleftharpoons ES \rightleftharpoons P + E$



## B7.5 – Problems with Lock/Key

- The lock and key model does not fully account for the combined events of binding and simultaneous chemical change observed in some enzyme-catalyzed reactions.
- It also fails to account for the broad specificity of some enzymes (some can bind to several different but related substrates).
- Some conformational changes in the shape of the active site occur during the formation of the ES complex



## B7.5 – Induced Fit Model

- To make up for these lacks in the Lock/Key model, the Induced Fit Model is used to demonstrate the important conformational changes in the active site when exposed to the amino acids of the substrate
- The analogy is that of a hand slightly changing the shape of a glove as the glove is put on.



# B7.5 – Induced Fit Changes

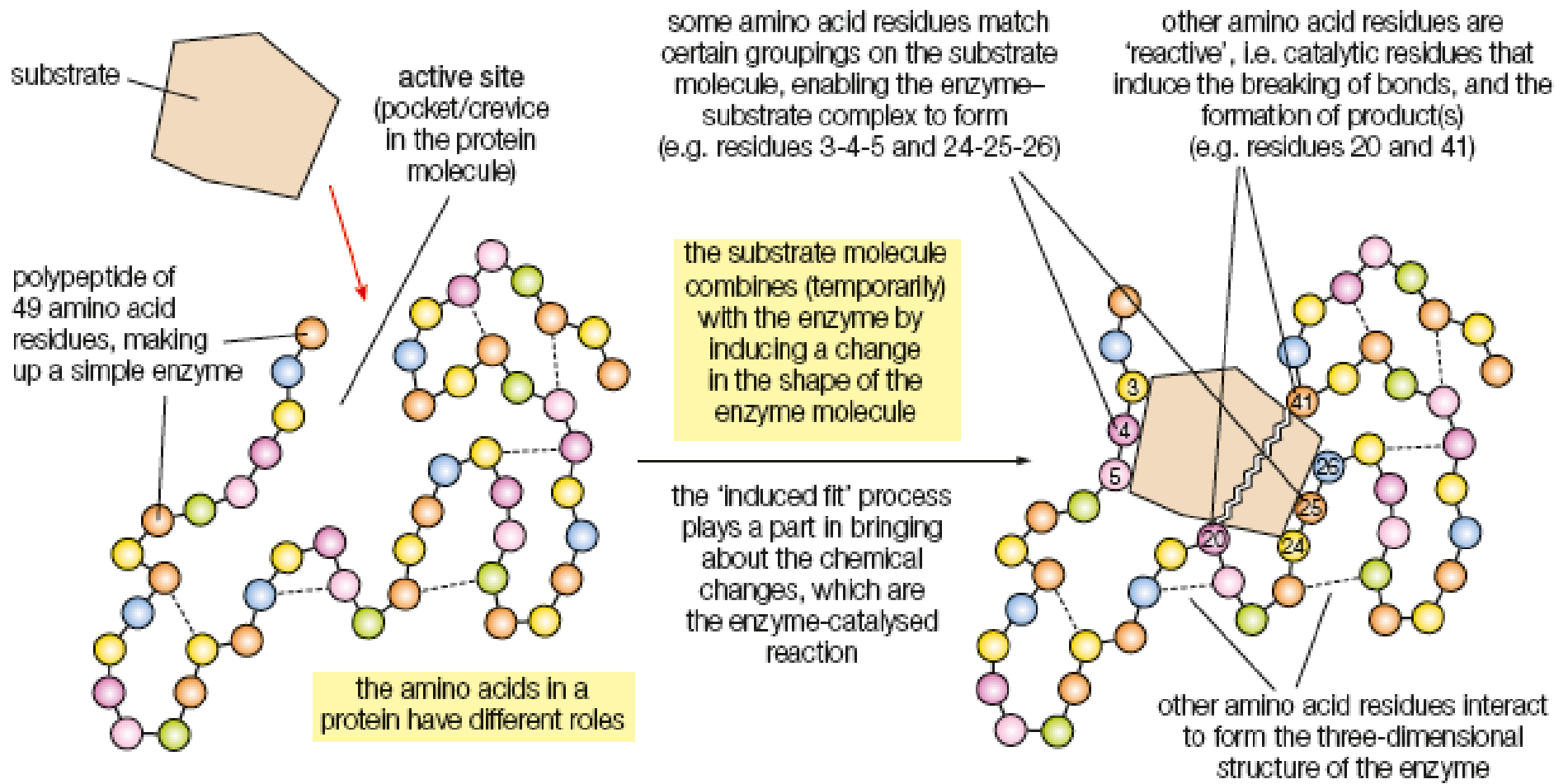


Figure 22.91 The induced fit model of enzyme action

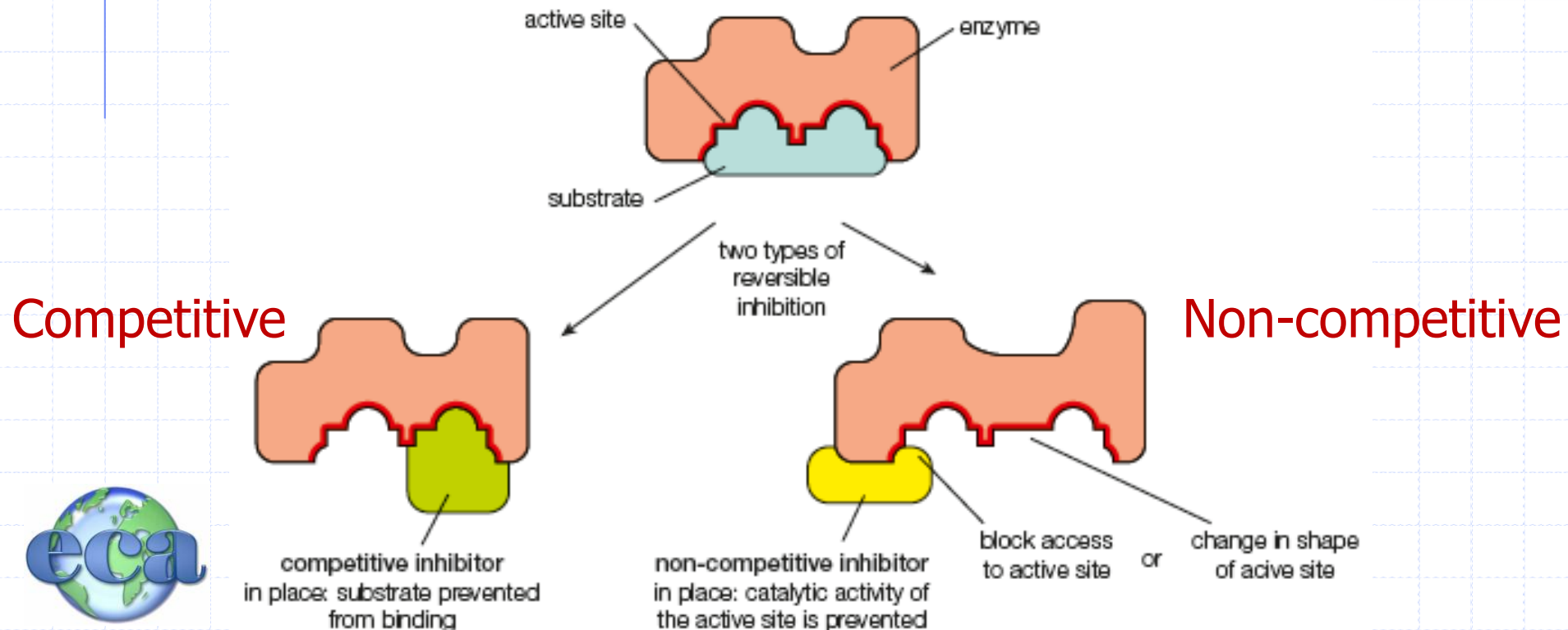
## B7.6 – Inhibition (Competitive & Non-Competitive)

- *B.7.6 Compare competitive inhibition and non-competitive inhibition. (3)*
- Going back to the lock and key model we can demonstrate the difference between the structure, specificity, and effect of competitive versus noncompetitive inhibitors



# B7.6 – Lock and Key

- The lock and key (ES) specificity and activity can be reduced by the presence of **inhibitors**
  - Competitive and non-competitive



## B7.4 – Competitive Inhibitors

- Competitive inhibitors resemble substrates sufficiently well to form some proper interactions with the active site
- Increased concentration of substrate would help in overcoming the presence of the inhibitor and  $V_{\max}$  can still be reached
- The extent of a competitive inhibitor depends on:
  - The concentration of the inhibitor
  - The concentration of the substrate
  - The relative affinity of the active site for the inhibitor and substrate



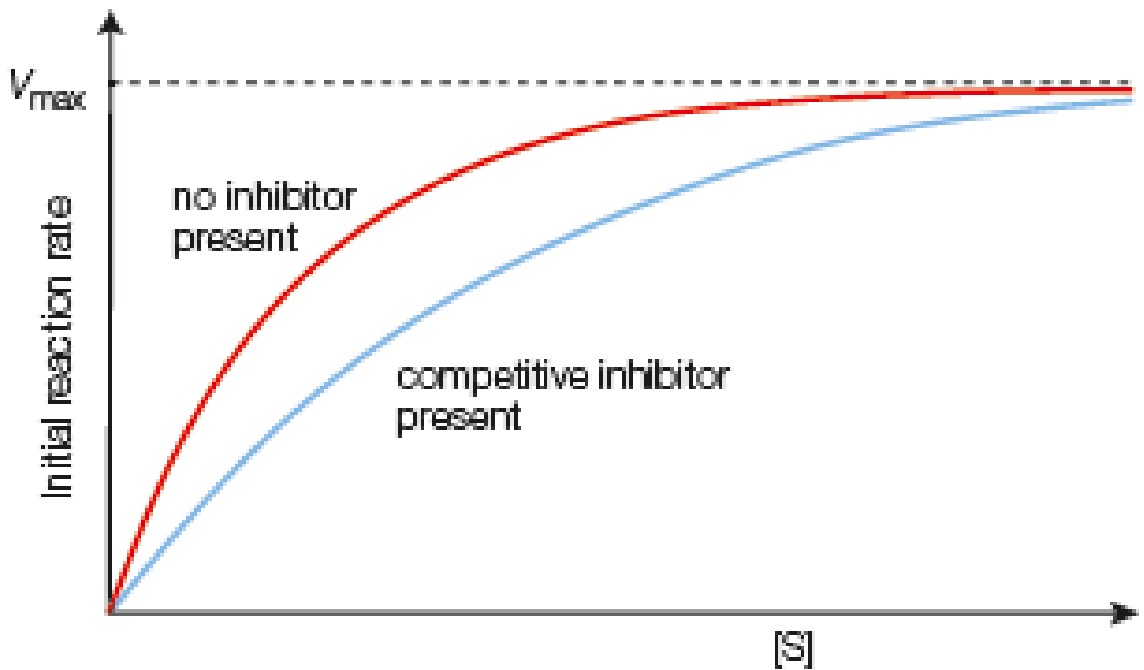
## B7.4 – Non-competitive Inhibitors

- Non-competitive inhibitors do NOT resemble the substrate but rather bind to a site on the enzyme other than the active site.
  - This often deforms the enzyme
  - Prevents access of the substrate to the enzyme
  - It can also bind with the ES complex
  - $V_{\max}$  is decreased no matter  $[S]$
  - Non-competitive inhibition depends on
    - ◆ The concentration of the inhibitor
    - ◆ The affinity of enzyme for the inhibitor

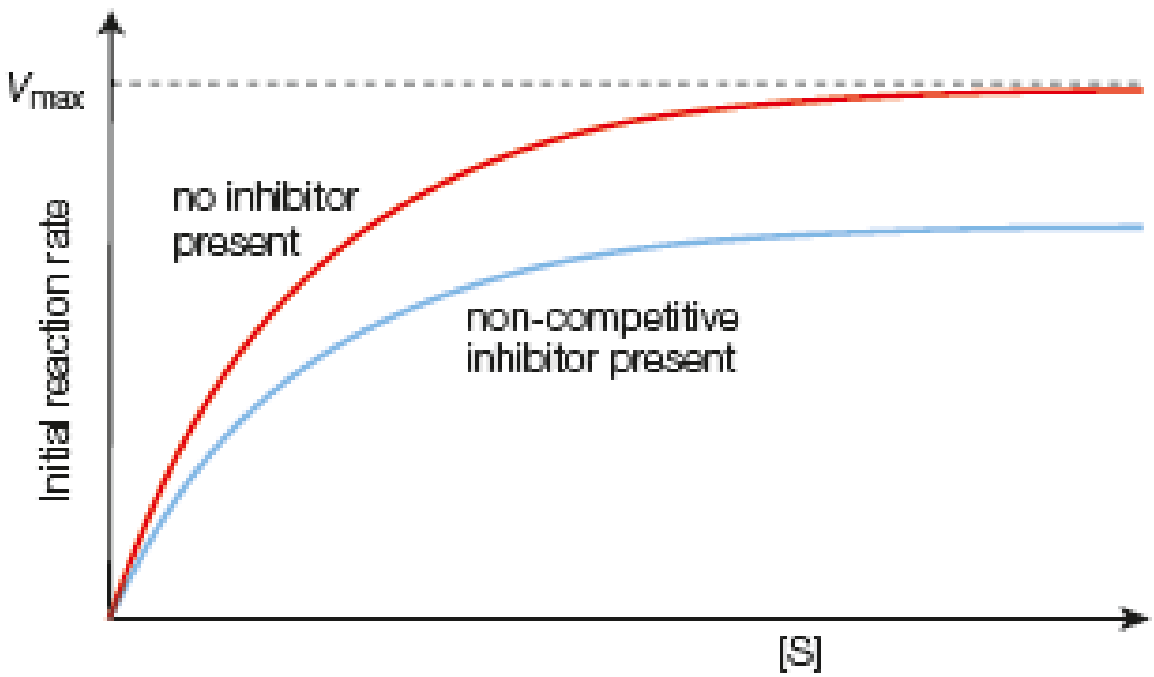




- **Competitive** inhibitors can be overcome by increased [S]
  - Resemble S



- **Non-competitive** interfere no matter the [S]



Do NOT resemble S

# B7.7 – Effects on Enzyme Activity

- *B.7.7 State and explain the effects of heavy metal ions, temperature changes and pH changes on enzyme activity. (3)*
  - Temperature
  - Heavy-metal ions
  - pH



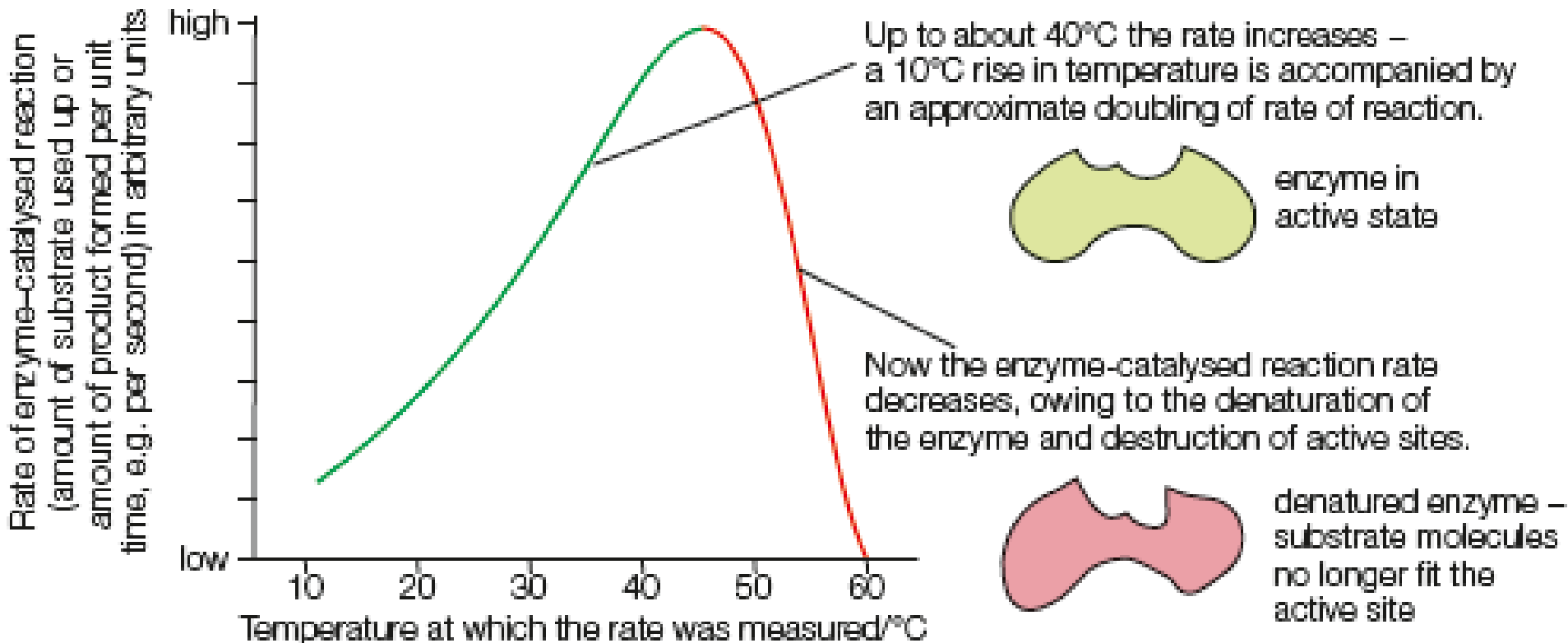
## B7.7 – Factors (Temperature)

- Increases the number of effective collisions of molecules as kinetic energy is increased
- Initially the rate of the reaction increases exponentially with increasing temperature until a maximum rate is achieved
  - Beyond the max rate, the reaction decreases, often rapidly and this loss of activity is not reversible
- Enzyme activity depends on conformation, temperature may interfere with weak intermolecular forces necessary for the enzyme.
  - May cause enzymes to uncoil and lose function



# B7.7 – Factors (Temperature)

- The effect of temperature on enzyme activity is apparent
  - Increase T will speed reaction until conformational changes are caused in the enzyme



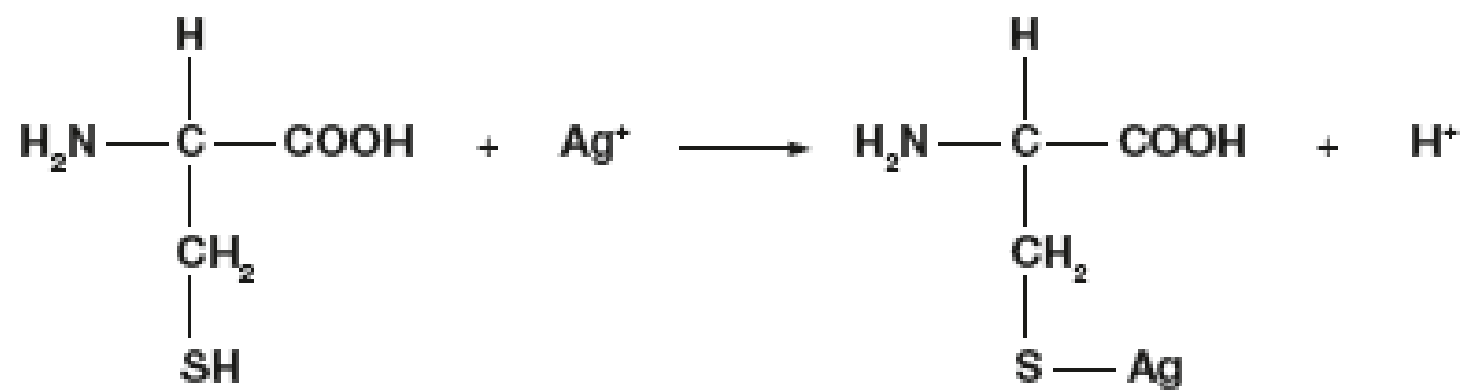
## B7.7 – Factors (Heavy M ions)

- The heavy metal ions (transition metals, lanthanides, actinides, some metaloids) are metals with a relatively high atomic mass.
  - Ex: Mercury, Cadmium, Zinc, Silver
  - At low concentrations can act as irreversible inhibitors (non-competitive) at low concentrations
  - They form bonds with free –SH groups present in the amino acid cysteine



# B7.7 – Factors (Heavy M ions)

- The free –SH groups, if present in the active site, may be essential to the activity of the enzyme
- Heavy metal ions may interfere with this functional group (often cysteine).



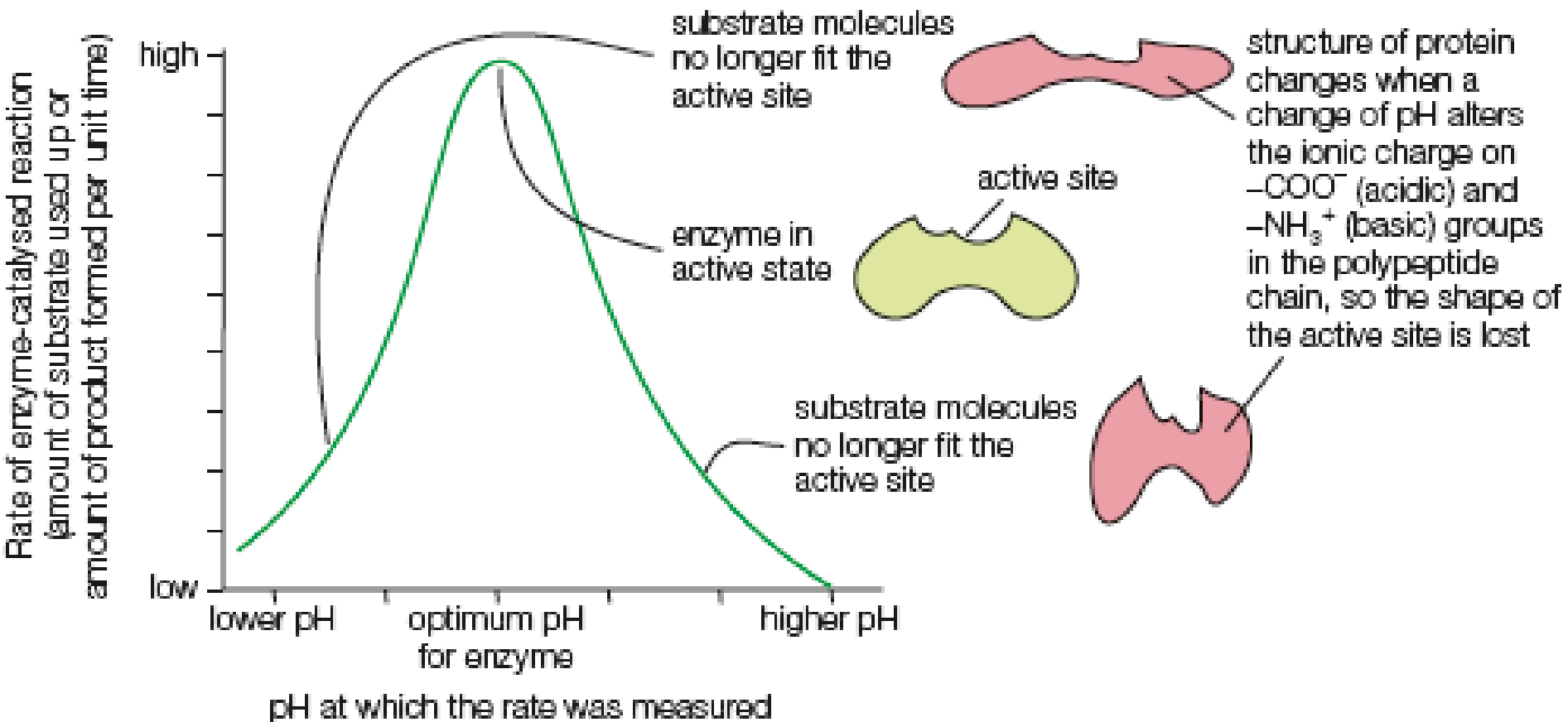
## B7.7 – Factors (pH)

- Many enzymes work efficiently over only narrow pH values.
- The optimum pH is that at which the maximum rate of reaction occurs (for many, pH 7)
- When pH value is above or below optimum, activity is significantly decreased
- Changes in pH alter the change of the active site
  - the acidic ( $-\text{COO}^-$ ) group
  - the basic ( $-\text{NH}_3^+$ ) group



# B7.7 – Factors (pH)

- Small changes in pH may interfere with amino acid conformation. Buffers are used as a solution





# B – Applications of Enzymes

## Applications of Chemistry

Application	Enzymes employed	Uses
Biological washing powder	Primarily proteases, produced in an extracellular form from bacteria	Used for pre-soak conditions and direct liquid applications, helping with removal of protein stains from clothes
Dishwasher detergent	Amylase enzymes	Detergents for machine dishwashing to remove resistant starch residues
Baby foods	Trypsin (a protease)	To pre-digest baby foods
Fruit juices	Cellulase, pectinase (act on cell walls)	Clarify fruit juices, e.g. apple juice
Baking industry	Fungal $\alpha$ -amylase enzymes: normally inactivated at about 50°C; destroyed during baking process	Catalyse breakdown of starch in the flour to glucose; yeast action on sugar produces carbon dioxide
Starch industry	Glucose isomerase	Converts glucose into fructose (high-fructose syrups derived from starchy materials have enhanced sweetening properties and lower calorific values)
Photographic industry	Protease	Dissolve gelatin off the scrap film, allowing recovery of silver present
Brewing industry	Enzymes from barley are released during the mashing stage of beer production	Degrade starch and proteins to produce simple sugars, amino acids and peptides that are used by yeast to enhance fermentation
Mouthwash (Figure 22.89)	Glucose oxidase, lactoperoxidase, lactoferrin and lysozyme	Contains four natural antibacterial enzymes that kill bacteria found in oral infections and gingivitis without any side-effects

Table 22.9 Industrial uses of enzymes



Figure 22.89 Biotene mouthwash